Genetic Limitations in the Exploitation of Base Populations of *Eucalyptus globulus* ssp. *globulus*

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Abstract

Large base population collections of *Eucalyptus globulus* ssp. *globulus* from native stands in Australia are currently being exploited for breeding in many countries. Constraints to the accurate estimation of genetic parameters and prediction of breeding values from these OP (open-pollinated) progenies are addressed. *E. globulus* ssp. *globulus* exhibits marked inbreeding depression for growth and the relative level of non-additive genetic variance appears high. Narrow-sense heritabilities for two-year growth estimated from OPs are highly inflated compared to controlled cross estimates from the same base parents. The correspondence between parental breeding value predictions for two-year growth from outcrossed versus OP progeny was poor. Site by genotype interactions also appear to be underestimated from OP progenies, possibly due to confounding environmentally sensitive, additive gene effects ('soft' effects) with the deleterious effects of inbreeding and non-additive genetic effects which are expressed relatively independent of the environment ('hard' effects).

Introduction

Extensive collections of OP (open-pollinated) seed have been made by the CSIRO Australian Tree Seed Centre from native stands of *Eucalyptus globulus* ssp. *globulus* and populations taxonomically intermediate between ssp. *globulus* and mainland subspecies (Gardiner and Crawford, 1987, 1988; Potts and Jordan, 1994). Trials have been established from these OP seed lots in many countries and selection is currently being undertaken to establish breeding and propagation populations (e.g. Jarvis *et al.*, 1995; Zang *et al.*, 1995). It is thus important to identify the biological and genetic constraints to the accurate estimation of genetic parameters and prediction of breeding values from these OP families.

Problems with open-pollinated progenies

The main problem for determining the genetic merit of this material arises from the uncertainty in the male pedigree. The classical approach in forestry is to treat siblings in families derived from open-pollination as having a half-sib relationship (coefficient of relationship, r = 0.25) (see Griffin and Cotterill, 1988; Borralho, 1994; Hardner and Potts, 1995). However, most eucalypt species undergoing domestication have a mixed mating system with estimates of the natural outcrossing rate averaging 74% (range 59-84%; Eldridge *et al.*, 1993; Table 19.2). *E. globulus* ssp. *globulus* appears to be no exception. Most trees will set at least some seed following artificial self pollination (Potts and Savva, 1988; Potts *et al.*, 1992; Hardner and Potts, 1994), although seed yield per flower crossed is usually substantially reduced compared to controlled outcrossing (74%, Hardner and Potts, 1994). While there is no evidence for selection against the products of self fertilisation at germination or in the nursery, the deleterious impact of selfing on subsequent growth is severe (Potts *et al.*, 1992; Hardner and Potts 1994). Inbreeding may be further exasperated in collections from native stands due to other forms of related matings, particularly due to neighbourhood inbreeding (Eldridge *et al.*, 1993, p. 196; Hardner *et al.*, 1995). The levels of inbreeding depression for growth and survival in OPs is on average intermediate between that of selfs and unrelated outcrosses (Hardner and Potts, 1994), clearly indicating that the cumulative, deleterious effects of the various types of related matings is marked.

Differential levels of inbreeding

Slight variations in the level of inbreeding may markedly alter performance at the individual, family and population level. In addition, individuals and populations may respond differentially to inbreeding depending on random variations in the genetic load of deleterious recessive alleles. In this 1987-88 CSIRO collection, OP seed was sampled from native stands from throughout the full geographical range of ssp. *globulus*, in order to capture as much of the genetic diversity as possible. This strategy necessitated sampling large, relatively continuous populations, remnant patches of forest or isolated trees in farmland, and small, naturally relic populations, which may differ in their levels of inbreeding.

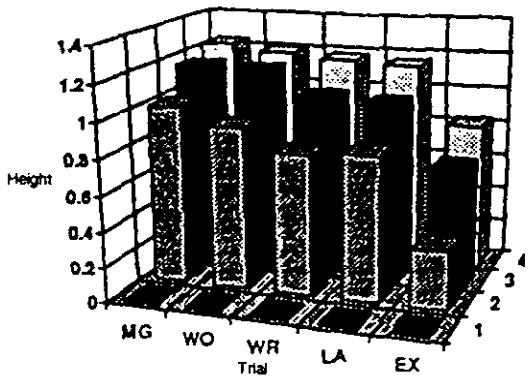


Figure 1. Estimates of the effect of stand structure surrounding parental trees on the height of 4 year old *E. globulus* ssp. *globulus* in five trials in Tasmania (expressed as the advantage over stand type 1; adapted from Borralho and Potts 1995). Trees in native stands were classified into four stand type categories - (1) isolated trees, (2) a few trees in a small isolated patch, (3) trees in open stands of continuous distribution and (4) trees in closed stands of continuous distribution. The effect of stand type in the model was highly significant (P<0.001).

Our studies have shown higher levels of seedling abnormalities and poorer growth of families derived from remnant populations in farmland compared to comparable families from relatively undisturbed, continuous populations, consistent with increased inbreeding (Potts and Jordan, 1994). Heritability estimates derived from the remnant stands are also highly inflated. Similar trends are also observed when stand type is used as an indicator of the number of potential pollinators surrounding each tree sampled in the collection (Borralho and Potts, 1995). As expected with decreasing inbreeding, there was a significant (P<0.001) increase in growth rate with decreasing isolation of parents at all sites examined (Fig. 1). Heritability estimates for height and diameter were also reduced by 4 to 13% by accounting for stand type in genetic models. This reduction agrees well with results from simulated OP populations, where heterogeneous rates of selfing inflated heritabilities by between 7 and 13% (Borralho, 1994).

We are taking stand type into account in an attempt to improve our breeding value predictions (Jarvis *et al.*, 1995). However, these results have caused us to question the reliability of genetic parameter estimates and, more importantly, the accuracy of the ranking of this material for breeding purposes.

Comparison of genetic information derived from OP and controlled outcross progenies

A sub-set of a crossing program undertaken in 1987-88 by the CSIRO, Division of Forestry with *E. globulus* ssp. *globulus* and *E. nitens* (see; Volker *et al.*, 1994) was designed to compare genetic parameters and parental breeding values derived from progenies grown from OP seed collected from native stands with those derived from controlled outcrossing of the same base parents. In the case of ssp. *globulus*, OP seed and pollen were collected from 26 trees in native stands (16 from Taranna and 10 from King Island; no OP seed was obtained from 1 tree). The pollen was crossed to eight ssp. *globulus* females growing in a seed orchard to produce a nearly complete 26 x 8 factorial design comprising both intra- and interprovenance crosses. These full-sib and OP families were planted together on five sites across Australia (Bega, NSW; Flynn, Victoria; West Ridgley and Parkham, Tasmania; Manjimup, WA) using an incomplete block design with 3-5 tree line plots of each family (see Hodge *et al.*, 1995; Vaillancourt *et al.*, 1995).

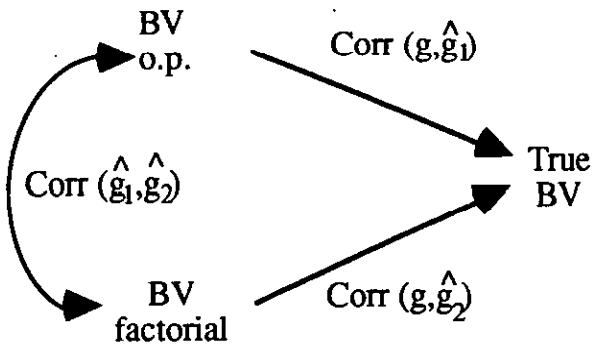
Heritability estimates

Our results have shown that heritabilities for two-year volume estimated from OP material of ssp. *globulus* from native stands are inflated, particularly in the across-sites analysis (Hodge *et al.*, 1995; Table 1). Similar results have been reported for frost resistance (Volker *et al.*, 1994). Various authors have attempted to correct OP heritability estimates from ssp. *globulus* by using a coefficient of relationship ranging from 0.3 (Borralho *et al.*, 1992), 0.4 (Volker *et al.*, 1990) to 0.54 (Woolaston *et al.*, 1991), as opposed to 0.25. In the present case, even assuming a coefficient of relatedness of 0.5 is still insufficient to avoid inflated heritability estimates from OP progenies. The within plot variation was also

Table 1. Estimates of heritability for 2-year conic volume from single site analyses and across sites for different populations of *E. globulus* (GOP = native stand open-pollinated, GXGC = intraprovenance crosses and GXGW = interprovenance crosses). Heritabilities estimated from OP progenies (GOP) assume a coefficient of relatedness, r = 0.5 and were highly inflated except at Bega. Sites correspond to, 1 = Bega, 2 = Parkham, 3 = Flynn, 4 = West Ridgley, 5 = Manjimup. Provenance differences were not marked at this age and have been included in the variation between parents. The genetic correlation amongst sites is also indicated (r_{B_g}). (From Hodge *et al.*, 1995).

	Site					Mean	Across	
Population	1.	2.	3.	4.	5		Sites	rBg
GOP	0.00	0.18	0.30	0.53	0.27	0.26	0.33±0.15	1.00
GXGC	0.15	0.12	0.14	0.23	0.16	0.16	0.08±0.01	0.54
GXGW	0.14	0.04	0.18	0.04	0.12	0.10	0.02±0.01	0.21

Figure 2. Correlations amongst predicted and true breeding values (BV).



consistently inflated in OP progenies (see also Hardner and Potts, 1994), no doubt due the presence of both depressed inbreds and vigorous outcrosses within the same OP family.

Prediction of breeding values

The correlation between breeding value predictions derived from open-pollination and pedigreed outcrossed material is a key issue (Fig. 2). This correlation will depend on numerous factors including the inheritance of the trait, its susceptibility to inbreeding depression, the genetic architecture of the species or population, and pollen environment, as well as sampling accuracy.

The breeding values of the ssp. *globulus* pollen parents predicted from their OP progenies (native stands) were poorly correlated with breeding values predicted from the intraprovenance (r=0.13, P=0.31, n=25) and interprovenance (r=0.21, P=0.51, n=25) crossing. This poor correlation may be due to (i) low heritability, (ii) poor correlation between the predicted breeding values and the unknown true breeding value (i.e. poor accuracy of the predictions themselves) and (iii) failure of the genetic assumptions (i.e. OP families are true half-sibs and that there are no maternal or non-additive genetic effects). The correlation between breeding value predictions derived from intra- and interprovenance crossing was significant (r=0.54, P=0.001, n=33) and provides an empirical estimate of the repeatability of breeding value predictions from controlled crossing. This high correlation obtained suggests that the poor OP correlation is due to violation of the genetic assumptions, rather than simply sampling error. Poor correlations between OP and controlled cross predictions have similarly been reported for four year growth traits in an independent study of ssp. *globulus* (Hardner and Potts, 1994), but better correlations have been observed for growth traits in *E. regnans* (Hardner and Potts, 1995) and *E. nitens* (Hodge *et al.*, 1995).

Genotype x environment interactions

The relative magnitude of GxE is a key factor in evaluating whether breeding programs should be regionalised (Burdon, 1977; Matheson and Raymond, 1984). All studies to date have utilised OP progenies to assess the magnitude of GxE in *E. globulus* (Volker and Orme, 1988; Woolaston *et al.*, 1991). The general conclusion has been that while GxE may be statistically significant, these effects are not of sufficient magnitude to warrant regionalisation of breeding programs, at least within Australia (e.g. Kube *et al.*, 1995; Borralho *et al.*, 1995). However, we have some evidence to suggest that OP based estimates may underestimate the importance of GxE, particularly the interaction of additive genetic differences with the environment (i.e. σ^2_{AE}). In the present study, the genetic correlation between sites ($r_{Bg} = \sigma^2_A/(\sigma^2_A + \sigma^2_{AE}$; Burdon, 1977) was unity for the OP population, but 0.54 and 0.21 for the intra- and interprovenance crosses respectively (Table 1). Not only is the variation between OP families unrelated to additive

Table 2. The significance of random effects and their variance components (s.e.) estimated for two-year volume from ssp. *globulus* factorial and OP progenies from the same base male parents. The analyses were undertaken with the MIXED procedure of SAS using plot means and ignoring provenance differences amongst parents. Site was treated as a fixed effect and incomplete block within site as a random effect. The significance of the random effects is based on the Wald Chi-squared test. The within plot variance was estimated in a separate analysis.

	Factorial				Open-pollination			
Effect	**df**	**σ^2 (x100)**	**s.e. (x100)**	**Prob**	**df**	**σ^2 (x100)**	**s.e. (x100)**	**Prob**
Female	7	2.49	1.51	0.099				
Male	25	0.15	0.27	0.585	24	12.93	4.45	0.004
Female x male	139	1.68	0.42	0.000				
Female x site	28	0.72	0.29	0.015				
Male x site	100	1.13	0.32	0.001	75	0.00	-	-
Female x male x site	281	0.00	-	-				
Residual	1754	17.98	0.56		467	26.14	2.46	
Within plot		40.28				62.28		
Between plot		8.22				10.24		
Phenotypic variance		54.66				85.45		

genetic differences between the parents, but these differences are consistently expressed whereas additive genetic differences between the parents are clearly dependent on the planting environment (e.g. male x site $P < 0.001$; Table 2). In contrast, the significant dominance variation (e.g. male x female; Table 2) was expressed independent of site (female x male x site; Table 2). The underestimation of GxE with OPs is believed to be due to the variation between OP families confounding environmentally sensitive, additive gene effects ('soft' effects) with large effects of deleterious, recessive genes which are exposed by inbreeding, and are expressed relatively independent of the environment ('hard' effects). Once differences in inbreeding levels are removed in advanced generations, it is possible that the relative importance of GxE will be increased well beyond that detected in the base population.

Conclusion

Additive genetic gains in growth estimated from OP progenies collected from native stands of ssp. *globulus* may be overestimated. The discrepancy may not be as large with traits of higher heritability (e.g. wood properties), but the importance of monitoring realised genetic gains is clear. Significant gains in growth may still be made simply through removal of inbreeding effects in subsequent generations by either controlled pollination or seed orchard establishment. In the present case, the average conic volume of the open-pollinated population was 17 and 13% less than the population of inter- and intraprovenance crosses respectively. The problems identified with OP progenies of ssp. *globulus* are likely to be of less importance in advanced generations (e.g. seed-orchards) where inbreeding levels amongst families may be more uniform and neighbourhood inbreeding removed. While these are early results, they suggest that strategy decisions using genetic parameters determined from base populations should be made cautiously and robust options adopted. Selection from OP base populations may lead to sub-optimal gains as poor performance under open-pollination may mask a high breeding value of a parent or individual, the breeder may be partially compensated by reduced costs, shorter generation intervals, indirect selection of self-sterility and purging of deleterious recessive genes. Nevertheless, there is a strong argument for reduced selection pressure in OP base populations, maintenance of a high proportion of the families originally sampled, and the rapid adoption of assessment through controlled pollination programs. Increased sophistication in statistical methodology for predicting breeding values will be wasted without a corresponding increase in the precision of genetic interpretation through full pedigree control.

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